

REMARKS

The application has been amended in a manner believed to place it in condition for allowance at the time of the next Official Action.

Claims 1-10 and 12-14 are amended. Claims 11, 15 and 16 are cancelled. Claims 17-22 are new. Claims 1-10, 12-14 and 17-22 remain pending.

Support for the amended and new claims may be found generally throughout the specification and specifically at page 2 lines 12-25, page 6 lines 27-29, page 7 lines 28-36, page 8 lines 14-36, page 9 line 30 to page 10 line 25, page 11 line 27 to page 12 line 17, page 13 lines 28-34, page 14 lines 10-15, page 23 line 15 to page 25 line 10, page 29 line 1 to page 30 line 3, page 31 line 19 to page 33 line 10, Example 4 and Example 7.

Applicants submit a substitute sequence listing to comply with the formal requirements relating to patent applications containing nucleotide sequences and/or amino acid sequence disclosures (e.g. descriptions of the artificial sequences).

Accordingly, a new sequence listing is attached to the present amendment, in paper and disc formats. Applicants hereby state that the attached paper and computer readable copies have the same content, and introduce no new matter into the present application.

The Official Action noted that an English translation of French Application 97/06977 had not been filed. A certified English translation is provided herewith attached to the present amendment.

Claims 1, 8, 10, 12, 15 and 16 were objected to for informalities.

Claims 1, 10, 12, 15, and 16 recited "quantic" for the fluorescent yield. Applicants acknowledge with appreciation the suggestion made by the Examiner to recite "quantum" instead of "quantic". Claims 1, 10 and 12 are amended as suggested by the Examiner and recite "quantum". Claims 15 and 16 are cancelled.

Claim 8 was objected to for omitting "of" after "consisting". Claim 8 is amended to recite "consisting of".

Therefore, applicants respectfully request that the objections be withdrawn.

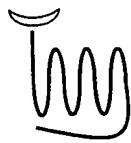
Applicants believe it would helpful to explain the present invention. The present subject matter is directed to a process for detecting and/or quantifying non-covalent interactions between a target receptor and one its ligands. This is a Fluorescence Energy Transfer (FRET) based process (page 7 lines 28-36). The principle of FRET is as follows (membrane-bound receptor as an example):



GFP1



GFP2



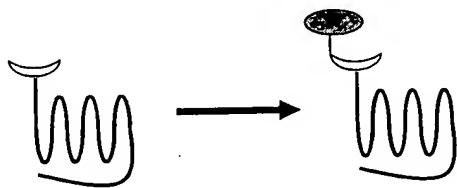
target receptor



ligand

Preparation of the fluorescent target receptor

In the receptor's DNA sequence, one or more amino acids are substituted, inserted or deleted and fused with the DNA coding for a GFP or a GFP mutant (genetic recombination). This will result in the expression of a target receptor-GFP1 hybrid.



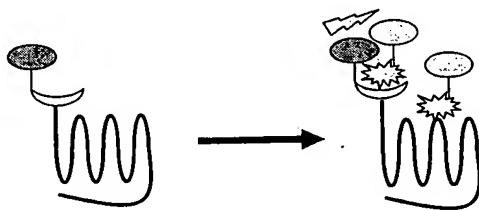
Preparation of the fluorescent ligand

In the potential ligand's DNA sequence, one or more amino acids are substituted, inserted or deleted and fused with the DNA coding for another GFP or GFP mutant (genetic recombination). The labeling can also be done by another substance (chemical binding). In our example, this will result in the expression of a ligand-GFP2 hybrid. In order to have a chance to find potential ligands, a whole library of ligand-GFP2 hybrids will be synthesized and screened on the target receptor-GFP1 hybrid.



After screening of a ligand library

FRET between GFP1 and GFP2 occurs only if the ligand is in the site of interest (which was labeled accordingly). GFP1 and GFP2 have different excitation spectra. When they are close to each other and excited according to the appropriate wavelength, one is the energy donor and the second is the energy acceptor according to their respective natures. The resulting FRET signal is detected using well known fluorimetry systems and techniques.



The invention allows the detection of non-covalent interactions between a target receptor and its ligand but also the measurement of fluorescence according to the nature of the interaction (the closest, the best energy transfer). The process of the invention is non-polluting (no radioactivity), cost effective (visible light-no quartz) and does not require any filtration step (page 3 lines 2-4).

Green Fluorescent Proteins (GFPs)

The term GFPs refer to a family of autofluorescent proteins derived from bioluminescent marine organisms. GFPs comprise a family of evolutionarily related proteins found in animals such as jellyfish and corals that belong to the phylum

Cnidaria (stinging aquatic invertebrates). These organisms have evolved GFPs as a part of a mechanism that enables them to glow in dark ocean waters. In recent years, scientists have begun to exploit GFP as a tool to explore the internal workings of living cells. The advent of GFP technology has revolutionized cell biology by enabling researchers to visualize proteins within living cells without the need for chemical staining.

Aequorea Victoria Green Fluorescent Protein (AvGFP) is one of a family of fluorescent proteins. AvGFP is a 238 amino acid, 27,000 Dalton monomer, it is intrinsically fluorescent and does not need any additional substrate or co-factor for fluorescent expression of the protein. This is an advantage over fluorescent dyes where the protein must be labeled and then delivered to the cell in a fluorescent form, or luciferases which require substrates. When introduced into cellular DNA and expressed as a fusion product with a specific protein, the location and translation of that protein may be tracked, due to the intrinsic fluorescence of the fluorescent protein.

The structure of GFPs allows them to be grafted onto other polypeptides, either on the amino-terminal (N-ter) or on the carboxy-terminal end without adversely affecting either its level of expression, or the formation of the fluorophore (fluorescent protein).

Thus, for one skilled in the art, the term GFPs refers to a family of protein and not to only one green fluorescent protein (e.g. wtGFP as illustrated in Fig. 1 or EGFP).

The Official Action rejected claims 12-14 under 35 U.S.C. 112, first paragraph, as allegedly failing to comply with the written description requirement.

Claims 12-14 are amended and do not recite "...buffers and media required for an energy transfer..."

Therefore, applicants respectfully request that the written description requirement rejection be withdrawn.

Claims 1-10 and 12-16 were rejected under 35 U.S.C. 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicants respectfully disagree.

Applicants believe it would be helpful to clarify the terms in the specification and in the claims.

The "target proteins" as recited in the claims refer to various types of receptors as shown in the detailed list of the "target proteins" (pages 14 line 25 to page 16 line 15). These receptors can be:

membrane-bound G protein coupled receptors (GPCR)

growth factor receptors

ion channel receptors

intracellular nuclear receptors

Accordingly, the claims are amended to recite "target receptor", instead of "target protein", to clarify the claimed subject matter.

The idea of the applicants was to use FRET technology between GFPs-labeled receptors and labeled ligands on membrane-bound receptors (with well defined binding sites) but also on nuclear receptors. Usually, the membrane-bound receptors have an N-ter extracellular domain carrying a part or the whole ligand binding site (the N-ter and C-ter domains are not close to one another). The nuclear receptors have N-ter and C-ter domains which are not fixed (so they can be close to one another). Accordingly, applicants show that the process of the invention can be used with a fluorescent protein being fused by its N-terminal side on the C-terminal side of the target receptor (amended claim 6) or by its N-terminal side on the C-terminal side of the target receptor (amended claim 5). Both claims are amended to clarify the claimed subject matter.

Claims 1, 10 and 16 were allegedly unclear because it was not clear how "a cell fragment can express a fluorescent protein fused with a nucleic acid encoding a target protein".

Applicants respectfully submit that the claims are directed to detecting an interaction between a membrane-bound receptor and one of its ligands. Functioning membrane-bound receptors are bound to either a complete cell or only fragments

of cells. These receptors can express a fluorescent or different fluorescent proteins.

As claimed, cell or cell fragments are prepared containing a DNA sequence comprising the gene coding for a fluorescent protein fused with the gene for the target membrane-bound receptor. It is important that the fusion between the two sequences does not modify the properties of the target receptor, including receptor-ligand interaction and transduction signal.

After preparing cells with the fused sequences, cell fragments may be prepared, as exemplified at page 49 line 35 to page 50 line 6. In this example, the cells are prepared in order to express the target receptor fused to a fluorescent protein. Then the cells are "broken" by enzymes to obtain cell fragments that express the target receptor fused to a fluorescent protein.

Thus, applicants believe that the specification discloses how "a cell fragment can express a fluorescent protein fused with a nucleic acid encoding a target protein", and the recitation in the claims is clear.

Claims 1, 10 and 12-16 were allegedly vague concerning the term GFP and "a fluorescent substance".

Claims 15 and 16 are cancelled.

For clarification of GFP, amended claims 1, 10 and 12 recite "...the fluorescent protein being chosen from fluorescent proteins obtained or derived from autofluorescent proteins of cnidarians, the molar extinction coefficient of which is greater

than about 14,000 M-1cm-1 and the quantum fluorescence yield is greater than about 0.38...". New claim 18 recites the selection of the fluorescent protein between GFP, EGFP, CFP or ECFP, YFP or EYFP and GFPUV.

Additionally claims 1, 10 and 12-14 are amended to clarify the term "fluorescent substance". The receptor is coupled to GFP or one of its mutants (page 3, lines 15-20) to maintain its structural and functional properties. The potential ligand is coupled to any fluorescent substance (GFP, mutants of GFP or other chemical compounds) in which "the fluorescent group is either a donor of energy to GFP or one of its mutants, or an acceptor of energy from GFP or one of its mutants" (page 3, lines 24-25). The term "a fluorescent substance" is used to mark the difference between the label of the receptor (GFP or GFP mutant) and the label of the potential ligand (GFP, GFP mutants or other chemical compounds). The only constraint for the ligand labels is their potency to excite or be excited by the label of the receptor (= efficient FRET reaction). The list of potential labels for the ligands is detailed on page 11, line 28 to page 29, line 18.

Thus, the terms GFP and fluorescent substance are not vague, as their meanings are defined by the specification.

Claim 1, 12 and 15 were allegedly unclear as to the language "selected from the group consisting of green fluorescent protein" and "chosen from green fluorescent protein".

Claim 15 is cancelled.

Claim 1 has been amended, and new claim 17 clarifies the nature of the label of claim 1. Claim 12 recites the nature of the label in a manner similar to new claim 17. Additionally, new claim 18 clarifies the nature of the Green Fluorescent Proteins (GFPs) selected for the purpose of the invention.

Accordingly, applicants believe that the groups for selection of the label and green fluorescent proteins are clearly defined by the claims.

Claim 2 was allegedly vague with respect to the recitation of "the G-protein" and "steroid factor", and claim 2 included terms that allegedly lacked antecedent basis (e.g. "the G-protein", "the insulin receptor, and "the steroid factor").

Claim 2 now recites "...the target receptor is selected from... membrane-bound G protein coupled receptors (GPCRs)...". This is in line with the NC-IUPHAR nomenclature (for more details see <http://www.iuphar.org>) for receptors. Additionally, in line with the NC-IUPHAR nomenclature, claim 2 recites "...insulin-like growth factor (IGF) receptors...". Claim 2 no longer recites "steroid factor".

Thus, as claim 2 recites G protein coupled receptors and insulin like growth factors in a manner known to one skilled in the art, claim 2 is definite.

Claims 3 and 4 were rejected for reciting that the labeled substance is Bodipy or coumarin.

Claims 3 and 4 are amended in a manner consistent with the specification, and, thus, are no longer indefinite.

Claims 5 and 6 were rejected for reciting the fluorescent protein was fused on a terminal side and the target protein was fused on a terminal side, but the claims did not recite to what the protein were fused.

Claims 5 and 6 are amended to recite that the fluorescent protein is fused to the target receptor, and, thus, the claims are clear.

Claim 9 was rejected for allegedly lacking antecedent basis for the recitation of "the donor" and "the acceptor", and for allegedly being unclear with respect to parameters applied to meet the standard of similar pharmacological specificity.

Claim 9 is amended to recite "...the compound being a fluorescence energy donor and the compound being a fluorescence energy acceptor..." as detailed on page 8, lines 14 to 36 and on page 7, lines 28 to 36 of the specification. Claim 9 also recites "...can be abolished by the addition of a non-fluorescent substance having the same binding site...". Accordingly, applicants believe that claim 9 does not lack antecedent basis and is definite.

Claims 12-14 were allegedly contradictory for reciting a GFP in claim 12, an EYFP, EGFP or ECFP in claims 13 and 14, followed by the recitation "a fluorescent protein".

The claims 13 and 14 are amended to recite "the fluorescent protein", and, thus, are not contradictory.

Claims 12-14 were also rejected for including a recitation directed to buffers and media for an energy transfer. The amended claims do not recite buffers and media for an energy transfer.

Claim 13 allegedly lacked antecedent basis for "said second fluorescent protein enhanced cyan fluorescent protein". Amended claim 13 recites "a second fluorescent protein being enhanced cyan fluorescent protein" and does not lack antecedent basis.

Claim 14 allegedly lacked antecedent basis for "the G-protein". Claim 14 recites "a G-protein" and does not lack antecedent basis.

In view of the above, applicants believe that the currently pending claims distinctly claim the inventive subject matter.

Therefore, applicants respectfully request that the indefiniteness rejection be withdrawn.

The Official Action rejects claim 1, 15 and 16 under 35 U.S.C. 102(a) as allegedly being anticipated by Miyawaki et al. Applicants respectfully disagree.

As applicants provide a certified English translation of the priority document French Application 97/06977 with this

Amendment, the Miyawaki et al. publication does not qualify as prior art.

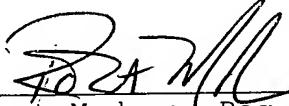
Therefore, applicants respectfully request that the anticipation rejection be withdrawn.

In view of the foregoing remarks, applicants believe that the application is in condition for allowance at the time of the next Official Action. Allowance and passage on that basis is therefore respectfully requested.

The Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 25-0120 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17.

Respectfully submitted,

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APPENDIX:

- Certified English translation of the priority document
- substitute Sequence Listing in paper and disk formats